EXHIBIT 5

Viral cultures for COVID-19 infectious potential assessment – a systematic review

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Summary

The reliability of RT-qPCR for assessing infectious potential of Covid-19 positives is defined by testing reference and culture specimens and their relation to patient characteristics (date and severity of symptoms, medical history) and test factors (cycle threshold).

ABSTRACT:

Objective to review the evidence from studies relating SARS-CoV-2 culture with the results of reverse transcriptase polymerase chain reaction (RT-PCR) and other variables which may influence the interpretation of the test, such as time from symptom onset

Methods We searched LitCovid, medRxiv, Google Scholar and the WHO Covid-19 database for Covid-19 to 10 September 2020. We included studies attempting to culture or observe SARS-CoV-2 in specimens with RT-PCR positivity. Studies were dual extracted and the data summarised narratively by specimen type. Where necessary we contacted corresponding authors of included papers for additional information. We assessed quality using a modified QUADAS 2 risk of bias tool.

Results We included 29 studies reporting attempts at culturing, or observing tissue infection by, SARS-CoV-2 in sputum, nasopharyngeal or oropharyngeal, urine, stool, blood and environmental specimens. The quality of the studies was moderate with lack of standardised reporting. The data suggest a relationship between the time from onset of symptom to the timing of the specimen test, cycle threshold (Ct) and symptom severity. Twelve studies reported that Ct values were significantly lower and log copies higher in specimens producing live virus culture. Two studies reported the odds of live virus culture reduced by approximately 33% for every one unit increase in Ct. Six of eight studies reported detectable RNA for longer than 14 days but infectious potential declined after day 8 even among cases with ongoing high viral loads. Four studies reported viral culture from stool specimens.

Conclusion

Complete live viruses are necessary for transmission, not the fragments identified by PCR. Prospective routine testing of reference and culture specimens and their relationship to symptoms, signs and patient co-factors should be used to define the reliability of PCR for assessing infectious potential. Those with high cycle threshold are unlikely to have infectious potential.

Keywords: Covid-19; mode of transmission, viral culture; symptom onset to test date; polymerase chain reaction; SARS-CoV-2; infectious potential.

Introduction

Effective prevention and management of SARS-CoV-2 infections relies on our capacity to identify those who are infected or potentially infectious. In the absence of predictive clinical signs or symptoms, the major means of detection is testing using Reverse Transcriptase quantitative Polymerase Chain Reaction (RT-qPCR) [1, 2, 3]

The test amplifies genomic sequences identified in specimens, and is highly sensitive, being capable of generating observable signals from specimens containing minute amounts of matching genomic sequence. Amplification of genomic sequence is measured in cycle thresholds (Ct), each cycle being a cut off for positive detection. There may be a correlation between Ct values from respiratory specimens, symptom onset to test (STT) date and positive viral culture. Evidence suggests the lower the Ct value and the shorter the STT, the higher the infectious potential. [4] If this is so, we should be able to identify those with the highest infectious potential.

Identification of a whole virion (as opposed to fragments) and proof that the isolate is capable of replicating its progeny in culture cells is the closest we are likely to get to a gold standard. [5] RT-qPCR cannot distinguish between the shedding of live virus or of viral fragments with no infectious potential, and it cannot measure the quantity of live virus present in a person's excreta. Although viral culture is difficult, time consuming and requires specialised facilities it potentially represents the best indicator of infection and infectious potential. We, therefore, set out to review those studies attempting viral culture, regardless of specimen type tested. We investigated the probability of successful culture with time from symptom onset to test and cycle threshold. We also examined the relationship between specimen cycle threshold and infectious potential.

Methods

We searched four databases: LitCovid, medRxiv, Google Scholar and the WHO Covid-19 database, using the terms 'viral culture' or 'viral replication' and associated synonyms on 10 September 2020. For relevant articles, citation matching was undertaken and relevant results identified.

We included studies reporting attempts to culture SARS-CoV-2 and those which also estimated the potential infectivity of the isolates or observed tissue infection by SARS CoV-2 and related them to other clinical variables such as date of symptom onset to test and patient characteristics.

Isothermal methods of detection are not included in our review, as they do not provide a Ct value

One reviewer extracted data for each study and a second reviewer checked the extraction. Heterogeneity and lack of detail of some of the reported data in the included studies prevented pooling. We tabulated data and summarised it descriptively by specimen: fecal, respiratory,

environment or mixed. Where possible, we also reported the duration of detectable RNA and the relationship of PCR cycle threshold and log 10 copies to positive viral culture.

Where necessary we contacted corresponding authors of the cited papers for additional information. We assessed quality using the QUADAS 2 risk of bias tool, simplified because the included studies were not designed as primary diagnostic accuracy studies. [6] Our methods are more fully described in our protocol (published on the 4th of July and updated on 5th of October 2020). [7]

Results

We identified 145 possible articles for inclusion and after screening, 29 full texts were read and included (see PRISMA [8] flow chart - Figure 1)**.** One unpublished study was not included as no permission was given by the authors. The included studies were published in 30 articles (see web appendix references w1-w29), four of which were in pre-print servers. The characteristics of each study are shown in Table 1. All included studies were case series of **moderate quality** (Table 2. Quality of included studies). We could not identify a protocol for any of the studies. All had been made public in 2020. We received five author responses regarding clarifying information (see Acknowledgments).

Studies using fecal specimens

Nine studies assessed viral viability from fecal specimens positive for SARS-CoV-2 based on RT-PCR result [W10, W11, W13, W17, W22, W23, W25-W27] One study reported infecting ferrets with stool supernatant; [w10] two reported visual growth in tissue [w19, w22[and four reported achieving viral replication [w13, w23, w24, w26]. In one further study, methods were unclear.[W28]

Studies using respiratory specimens

Seventeen studies reported attempting viral isolation and culture from respiratory specimens [W3, W4, W6-10, W13-16, W18, W21-23, W26, W27] One study successfully cultured 26/90 nasopharyngeal specimens: positive cultures were observed only up to day eight post-symptom onset; [w7] another study obtained cultures from 31/46 nasopharyngeal and oropharyngeal specimens. [w3] The largest study came from the La Scola group publications [w15] with positive cultures of 1,941 from 3,790 specimens. Another study of UK health care workers during a period of low viral circulation isolated SARS Cov-2 from 1/19 specimens. [w5]

Two more studies reported a clear correlation between symptoms onset, date of sampling, Ct and likelihood of viral culture. [w18, w21]

One study [w14] of nasopharyngeal specimens from 638 patients aged <16 years reported achieving culture from 12 (52%) of the 23 who tested positive for SARS CoV-2 with a Ct of around 28. Gniazdowski [w8] assessed RNA and infectious virus detection in 161 nasopharyngeal specimens from hospitalised Covid-19 patients. Positive culture was associated with Ct values of 18.8 ± 3.4 (median 18.7); negative culture was associated with mean Ct values 27.1 ± 5.7 (median 27.5). Over 90% of the virus isolates were obtained from specimens with a Ct value below 23

Basile [w4] reported 24% culture positivity, with specimens significantly more likely to be positive from ICU. A report by the Korean Centres for Disease Control failed to grow live viruses from 108 respiratory specimens from "re-positives" i.e. people who had tested positive after previously testing negative.[w12]

Ladhani [w16] and colleagues reported a successful culture rate of 87/158 RT-PCR positive naso-pharyngeal specimens from six nursing homes in London.

Studies using environmental specimens

Two possible (the text is unclear) positive cultures were obtained from 95 environmental specimens in one study that assessed aerosol and surface transmission potential of SARS-CoV-2 [w20]. No viruses could be grown from specimens from seven areas of a large London hospital from specimens with a cut-off RT-PCR Ct > 30. [w29]

Ahn and colleagues [w1] failed to grow live virus from an unspecified number of air specimens from isolation rooms of patients with severe Covid-19, but were able to grow virus from swabs of handrails, and the external surfaces of intubation cannulae.

Mixed sources

Some studies labelled as mixed source specimens are also reported by indvidual specimen in this text.

Eight studies reported viral culture from mixed sources: 12 oropharyngeal, nine nasopharyngeal and two sputum specimens [w9], one stool specimen and an unreported number of other specimens[w10], from saliva, nasal swabs, urine, blood and stool collected from nine Covid-19 and a possible

specimen stool culture [[w23], nine nasopharyngeal, oropharyngeal, stool, serum and urine specimens [w13], seven sputum specimens, three stool specimens and one nasopharyngeal specimen of 11 patients. [w26] . In this study all specimens had been taken within 5 days of symptom onset and there was a relationship between copy thresholds and cytopathic effect observed in infected culture cells.

Kim and colleagues reported no viral growth from an unclear number of serum, urine and stool specimens, despite these specimens being collected soon after admission [w11] . Lu and colleagues also reported no viral growth, however their specimens were from 87 cases tested "re-positive". [w17]

One study [w27] reported 21 positive cultures from from naso-pharyngeal specimens of 19 hospitalised patients in Singapore but no growth from specimens with a Ct value >30, or collected >14 days after symptoms onset. No culture was achieved from the urine or stool specimens.

Blood cultures

In one study by Andersson [w2] et al 20 RT-PCR positive serum specimens from 12 individual patients were selected at random from a Covid-19 specimen bank at 3 to 20 days following onset of symptoms. None of the 20 serum specimens produced a viral culture.

Post mortem study

One study on alveolar specimens from 68 elderly deceased reported postmortem studies on lung tissues from six cases were available for viral isolation. The evaluation showed viable SARS-CoV-2 in all six cases - in one case on day 26 from symptom onset. [w6]

Duration of RNA viral detection

Table 3 shows that nine studies report on the duration of viral RNA detection as assessed by PCR for SARS-CoV-2 RNA. [w7, w8, w10, w12, w13, w21, w24, w25, w27] All nine studies reported RNA detection for longer than 7 days. Young et al [w27] reported that SARS-CoV-2 was detectable from nasopharyngeal swabs by PCR up to 48 days after symptom onset.

Live viral culture window

The live viral culture time window was much shorter than for viral RNA identification, ranging from less than 8 days from symptom onset to test [w23] and Ct < 24 [w7]. Median duration of viral RNA identification in culture was 4 days (InterQuartile Range: 1 to 8) [w21].

The relationship between RT-PCR results and viral culture of SARS-CoV-2

Table 4 shows that ten studies analysed the relationship between Ct values and the possibility of culturing live virus [w4, w5, w7, w8, w9 w15, w16, w21, w23, w27] and three quantified the mean log copies of detected virus and live culture [w9, w14, w18]. All reported that Ct were significantly lower and log copies were significantly higher in those with live virus culture. Five studies reported no growth in specimens based on a Ct cut-off value [w5, w7, w9, w16, w27] ranging from CT > 24 [w7] to 35 [w15].

The estimated probability of recovery of virus from specimens with Ct > 35 was 8.3% (95% CI: 2.8% to 18.4%)[w21]. All donors above the Ct threshold of 35 (n=5) producing live culture were symptomatic.

In six London nursing homes there was no correlation between Ct values and symptoms in either residents or staff, [w16] although nearly 50% of both categories were asymptomatic.

One study [w9] reported different cut-off thresholds depending on the gene fragment analysed[34]. No growth was found for the NSP 12 fragment at Ct > 31.5, whereas the value was higher for the N gene fragment (>35.2).

The odds for culturing live virus decreased by 0.64 for every one unit increase in Ct (95%CI 0.49 to 0.84, p<0.001) [w7]; another study[w21] reported similar results in line with empirical evidence of an increased Ct of 0.58 per day since symptoms started. [9]

Discussion

The studies in this review attempted, and some successfully achieved, culture of SARS-CoV-2 in the laboratory, using a range of different specimens. There is evidence of a positive relationship between lower cycle count threshold, likelihood of positive viral culture and date of symptom onset. [10] This is seen clearly in the two studies assessing the infectious potential of "re-positives", i.e. COVID-19 cases who had been discharged from hospital after testing negative repeatedly and who then tested positive again after discharge: Lu 2020 [w17], Korean CDC [w12].

Lu and colleagues considered four hypotheses for the origin of "re-positives" [w17]. On the basis of their evidence they discarded re-infection and latency as explanations, and concluded that the most plausible explanations were either contamination of the specimen by extraneous material or

identification in the specimen of minute and irrelevant particles of dead SARS-CoV-2 representing virus long neutralised by the immune system.

Rapid expansion in testing capability requires training protocols and precautions to avoid poor laboratory practice which may not be possible in the time pressure of a pandemic. The evidence in this review shows that those with high cycle threshold are unlikely to have infectious potential.

Interpreting the results of RT-PCR requires consideration of patient characteristics such as symptoms and their severity, contacts history, presence of pre-existing morbidities and drug history, the cycle threshold value, the number of days from symptom onset to test and the specimen donor's age.[11 12]

Several of our included studies assessed the relationship of these variables and there appears to be a time window during which RNA detection is at its highest with low cycle threshold and higher possibility of culturing a live virus, with viral load and probability of growing live virus of SARS-CoV2 peaking much sooner than that of SARS CoV-1 or MERS-CoV.[11] We propose that further work should be done on this with the aim of constructing an algorithm for integrating the results of PCR with other variables, to increase the effectiveness of detecting infectious patients.

PCR should be continuously calibrated against a reference culture in Vero E6 cells in which cytopathic effect has been observed [w6]. Confirmation of visual identification using methods, such as an immunofluorescence assay may also be needed to aid diagnosis. [13] Henderson and colleagues have called for a multicentre study of all currently manufactured SARS-CoV-2 nucleic acid amplification tests to correlate the cycle threshold values on each platform for patients who have positive and negative viral cultures. Calibration of assays could then be done to estimate virus viability from the cycle threshold with some certainty. [14]

Ascertainment of infectious potential is all the more important as there is good evidence of viral RNA persistence across a whole range of different viral diseases with little or no infectious potential in the post infectious phase of MERS,[15] measles,[16] other coronoviridae, HCV and a variety of animal RNA viruses.[17]

In one COVID-19 (former) case, viral RNA was detectable until day 78 from symptoms onset with a very high Ct [18] but no culture growth, implying a lack of infectious potential.

SARS CoV-2 methods of cell culture vary and to our knowledge have not been standardised. Methods vary depending upon the selection of the cell lines; the collection, transport, and handling of and the maintenance of viable and healthy inoculated cells. [19] We therefore urgently recommend the development of standard culture methods and external quality assessment schemes for laboratories offering testing for SARS CoV2. [20 21] If identification of viral infectious potential relies on

visual inspection of cytopathogenic effect, then a reference culture of cells must also be developed to test recognition against infected cells. Viral culture may not be appropriate for routine daily results, but specialized laboratories should use viruses as controls, perform complete investigations when needed, and store representative clinical strains whenever possible. [22] Current evidence is too limited to establish the feasibility of generating a universal cycle threshold value as this may change with circumstances (e.g. hospital, community, cluster and symptom level), laboratory methods, so more information is urgently needed [23].

We suggest the WHO produce a protocol to standardise the use and interpretation of PCR and routine use of culture or animal model to continuously calibrate PCR testing, coordinated by designated Biosafety Level III laboratory facilities with inward directional airflow.[24] Further studies with standardised methods [25] and reporting are needed to establish the magnitude and reliability of this association.

The results of our review agree with the scoping review by Byrne and colleagues on infectious potential periods [26] and those of the living review by Cevick and colleagues[11]. The authors reviewed 79 studies on the dynamics, load and RNA detection for SARS CoV-1, MERS and SARS CoV-2 from symptoms onset. They concluded that although SARS-CoV-2 RNA identification in respiratory (up to 83 days) and stool (35 days) can be prolonged, duration of viable virus is relatively short-lived (up to a maximum of 8 days from symptoms onset). Those results are consistent with Bullard et al who found no growth in specimens with a cycle threshold greater than 24 [w7] or when symptom onset was greater than 8 days, and Wölfel et al [w23] who reported that virus could not be isolated from specimens taken after day 8 even among cases with ongoing high viral loads. The review by Rhee and colleagues reaches conclusion similar to ours.[10]

The importance of symptom onset and reported PCR threshold is shown in a study that collected test data during a prospective household transmission study. The authors found that Ct values were lowest soon after symptom onset and correlated with time elapsed since symptom onset (within 7 days after symptom onset, the median Ct value was 26.5 compared with a median of 35.0 21 days after onset). Ct values were significantly higher among those participants reporting no symptoms, and lower in those reporting upper respiratory symptoms at the time of specimen collection.[28]

The evidence is increasingly pointing to the probability of culturing live virus being related to the amount of viral RNA in the specimen and, therefore, inversely related to the cycle threshold. Thus, detection of viral RNA *per se* cannot be used to infer infectiousness. Duration of excretion may also be linked to age, male gender and possibly use of steroids and severity of illness.

Our review is limited by the lack of standardised reporting and lack of standard testing methods amongst the included studies[20]. Ct threshold reporting was inconsistent, preventing pooling or further in-depth analysis of the data, and insufficient clinical details were reported to define the possible role of asymptomatics or pre-symptomatics in transmission. The included studies were case reports or case series with a mixture of laboratory and clinical data, and variable in reporting the relation between donor characteristics and PCR results.

We may have missed some studies or new studies as they are published and we aim to update this review with emerging evidence.

Conclusion

The evidence gathered in this review points to a relationship between the time from collection of a specimen to test, cycle threshold, and symptom severity. We recommend that a uniform international standard for reporting of comparative SARS-CoV-2 culture with index test studies be produced. Particular attention should be paid to the relationship between the results of testing, clinical conditions and the characteristics of the source patients, description of flow of specimens and testing methods. Defining cut off levels predictive of infectious potential [27]should be feasible and is necessary for diagnosing viral respiratory infections using molecular tests.

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Disclaimer: The views expressed in this commentary represent the views of the authors and not necessarily those of the host institution, the UK NHS, the NIHR, or the Department of Health and Social Care. The views are not a substitute for professional medical advice. It will be regularly updated see the evidence explorer at https://www.cebm.net/evidence-synthesis/transmission-dynamics-of-covid-19/ for regular updates to the evidence summaries and briefs.

Potential conflicts:

Tom Jefferson is a Senior Associate Tutor and Honorary Research Fellow, Centre for Evidence-Based Medicine, University of Oxford. Disclosure statement is here

TJ was in receipt of a Cochrane Methods Innovations Fund grant to develop guidance on the use of regulatory data in Cochrane reviews (2015-018). In 2014–2016, TJ was a member of three advisory boards for Boehringer Ingelheim. TJ was a member of an independent data monitoring committee for a Sanofi Pasteur clinical trial on an influenza vaccine. TJ is occasionally interviewed by market research companies about phase I or II pharmaceutical products for which he receives fees (current). TJ was a member of three advisory boards for Boehringer Ingelheim (2014-16). TJ was a member of an independent data monitoring committee for a Sanofi Pasteur clinical trial on an influenza vaccine (2015-2017). TJ is a relator in a False Claims Act lawsuit on behalf of the United States that involves sales of Tamiflu for pandemic stockpiling. If resolved in the United States' favor, he would be entitled to a percentage of the recovery. TJ is co-holder of a Laura and John Arnold Foundation grant for development of a RIAT support centre (2017-2020) and Jean Monnet Network Grant, 2017-2020 for The Jean Monnet Health Law and Policy Network. TJ is an unpaid collaborator to the project Beyond Transparency in Pharmaceutical Research and Regulation led by Dalhousie University and funded by the Canadian Institutes of Health Research (2018-2022). TJ consulted for Illumina LLC on next generation gene sequencing (2019-2020). TJ was the consultant scientific coordinator for the HTA Medical Technology programme of the Agenzia per i Serivizi Sanitari Nazionali (AGENAS) of the

Italian MoH (2007-2019). TJ is Director Medical Affairs for BC Solutions, a market access company for medical devices in Europe. TJ is funded by NIHR UK and the World Health Organization (WHO) to update Cochrane review A122, “Physical Interventions to interrupt the spread of respiratory viruses”. TJ is funded by Oxford University to carry out a living review on the transmission epidemiology of COVID-19. Since 2020, TJ receives fees for articles published by The Spectator and other media outlets.

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Jon Brassey is the Director of Trip Database Ltd, Lead for Knowledge Mobilisation at Public Health Wales (NHS) and an Associate Editor at the BMJ Evidence-Based Medicine. Jon is a shareholder in Trip Database Ltd.

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Tables:

Table 1. Characteristics of included studies

Table 2. Quality of included studies

Table 3. Duration of viral detection

Table 4. Relationship of PCR Cycle threshold and Log 10 copies to Positive Viral Culture

Figures: Figure 1 PRISMA flow chart.

Table 1 Characteristic of Included Studies

Study [ref id]	Specimens (source)	Specimens (n) [SST]	Culture methods	Culture Positive	Additional notes
Ahn 2020 [W1]	Air and surfaces of isolation room of 3 patients with severe Covid 19	48 [not reported]	Only positive samples (Ct value ≤35 for the RdRp and E genes) were cultured in Vero E6 cells 10-fold dilutions of the SARS-CoV-2 supernatants from the environmental specimens was used. The inoculated cultures were grown in a humidified 37°C incubator with 5% CO2. After 72 hours, areas of cell clearance with crystal violet staining were used to demonstrate the cytopathic effect. In the presence of cytopathic effect was observed, detection of nucleic acid of SARS-CoV-2 by rRT-PCR in the supernatant was performed to confirm a successful culture.	External surfaces of intubation cannulae and surfaces in the room of patient not intubated	No air specimens grew virus Ct values of specimens who grew virus were uniformly low below 30 except in one case.
Andersson 2020 [W2]	20 RT-PCR positive serum specimens, selected at random from a Covid-19	20 serum specimens from 12 hospitalised	Specimens VC01-20 were provided blinded for viral culture experiments. 50 µL aliquots of specimens VC1-VC20 were separately added to 2.4 x 105 Vero E6 cells in 24-well plates.	0 / 20 these serum specimens produced positive viral culture	Serum specimens.

	specimen bank, representing specimens from 12 individual patients (four individuals were represented at two timepoints), collected at 3 to 20 days following onset of symptoms.	Covid-19 patients	Cells were propagated in DMEM supplemented with 10% FBS. Virus growth assays were done in DMEM supplemented with 1% FBS, glutamine and penicillin/streptomycin, according to published methods. In parallel, wells of the same number of cells were cultured in triplicate without virus challenge but with 50 µL control serum (VC21), or in duplicate with a stock of Victoria/01/2020 SARS-CoV-2 passage 4 (Oxford) at calculated ten-fold serial dilutions per well of 78, 7.8, 0.78 and 0.078 plaque forming units (pfu) in 50 µL of control serum (VC21). Wells were observed daily for cytopathic effects (CPE), and 50 L specimens were taken for vRNA extraction on day 3 post-challenge. On day 4, 50 L aliquots of supernatants from cells challenged with VC01-20 were "blind passaged" to fresh cells, and the remaining supernatants were harvested and stored separately at -80C for future analysis. After a further 3 days, CPE was recorded, if any, for second passage cultures.		
Arons 2020 [W3]	nasopharyngeal and oropharyngeal swabs	48 rRT-PCR–positive specimens [For asymptomatic median 4 days, Ct 23.1]	All rRT-PCR positive specimens shipped to USA CDC for viral culture using Vero-CCL-81 cells. Cells showing cytopathic effects were used for SARS-CoV-2 rRT-PCR to confirm isolation and viral growth in culture.	31 [no relation to symptoms presence. Culturable virus isolated from 6 days before to 9 days after symptom onset]	

Basile 2020 [W4]	234 specimens, 228 (97%) from the upper respiratory tract (sputum, naso pharyngeal swabs, bronchial lavage from 195 individuals with Covid-19.	Specimens from routine laboratory tests or from patients admitted to ICU or from a physician request [mean 4.5 days, 0-18, only one day to day 18]	Probes targets for PCR included E, RdRp, N, M, and ORF1ab for specimens from ICU patients and 1 to 4 E, RdRp, N and Orf1ab for all other specimens. After stabilization at 4 degrees centigrade specimens were inoculated into Vero E6 cells and incubated at 370C in 5% CO2 for 5 days (day 0 to 4). Cultures were observed daily for cytopathic effect (CPE). CPE when it occurred took place between days 2 and 4. Day 4 was chosen for terminal sampling.	Culture positivity rate was 56 (24%) and significantly more likely positive in ICU patients compared with other inpatients or outpatients and significantly more likely positive in specimens from inpatients	The highest Ct value with a successful culture was 32 (N gene target). A Ct cut-off of ≥37 was not indicative of viable virus
Borczuk 2020 [W5]	Post mortem lung tissue from 68 elderly deaths (median age 73)	Six	When a cytopathic effect was seen, the Vero cell culture supernatant was passed to a fresh Vero cell culture tube to ensure reproducibility. SARS-CoV-2 in the supernatant was further confirmed by RT-PCR	6	No ct reported. In one case virus grew on day 26 from symptoms kick off
Brown 2020 [W6]	Combined viral throat and nose swab from each participant n=1,152	Health care workers in six UK hospitals	Specimens were sent on the same day for detection of SARS-CoV-2 RNA by RT-PCR to the PHE national reference laboratory (five hospitals) or one hospital laboratory. The PHE laboratory used an Applied Biosystems 7500 FAST system targeting a conserved region of the SARS-CoV-2 open reading frame (ORF1ab) gene. The hospital laboratory used a different CE-IVD kit, targeting 3 SARS-CoV-2 genes (RdRp, E, and N). Both PCRs had	SARS-CoV-2 virus was isolated from only one (5%) of nineteen cultured specimens. It had a Ct value of 26.2.	Symptoms in the past month were associated with threefold increased odds of testing positive (aOR 3.46, 95%CI 1.38 to 8.67; p = 0.008). 23 of 1,152 participants

			internal controls. Viral culture of PHE laboratory positives was attempted in Vero E6 cells with virus detection confirmed by cytopathic effect up to 14 days post- inoculation.		tested positive (2.0%) with a median Ct of 35.70 (IQR:32.42 to 37.57).
Bullard 2020 [W7]	Nasopharyngeal (NP) or endotracheal (ETT) from COVID-19 patients (mean age 45 years)	90 [0 to 7 days]	NP swabs and ETT specimens in viral transport media were stored at 4°C for 24-72 hours until they were tested for the presence of SARS-CoV-2 RNA using real-time RT-PCR targeting a 122nt portion of the envelope gene (E gene). Dilutions were placed onto the Vero cells in triplicate and incubated at 37°C with 5% CO2 for 96 hours. Following incubation of 4 days, cytopathic effect was evaluated under a microscope and recorded.	26	The range of symptoms onset to negative PCT was 21 days. Within this period, positive cultures were only observed up to day 8 post symptom onset
Gniazdowski 2020 [W8]	161 probably nasopharyngeal specimens	161 cases with positive PCR [not reported]	Ct values were calculated of only one gene target per assay: the Spike (S) gene for the RealStar® SARS-CoV-2 and the nonstructural protein 101 (Nsp) 2 gene for the NeuMoDx™ SARS-CoV-2 assays. Genome sequencing was carried out. Incubation of the inoculum in VeroE6 cells cultured at 37°C was observed for 4 days for cytopathic effect and immunofluorescence used to identify viral presence	Unclear possibly 47 isolates	Positive culture was associated with Ct values of 18.8 ± 3.4. Infectious viral shedding occurred in specimens collected up to 20 days after the first positive result in symptomatics. Mean and 184 median Ct values associated with recoverable virus were 18.8 ± 3.4 and 18.17 respectively, which

					was significantly lower than the mean and median Ct values that did not correlate with infectious virus recovery: 27.1 ± 5.7 and 27.5 respectively. PCR results should be interpreted alongside symptoms
Huang 2020 [W9]	Oropharyngeal (OP) or nasopharyngeal (NP) swabs, or sputum (SP)	60 specimens from 50 cases [3,4 days mean but see table 1 for freeze thaw cycles delays]	SARS-CoV-2 cDNA was prepared using RNA extracted from the specimens of the first patient with confirmed COVID-19. RT was performed using the MMLV Reverse transcription kit. All procedures for viral culture were conducted in a biosafety level-3 facility. Vero-E6 and MK-2 (ATCC) cells were maintained in a virus culture medium and the cells were maintained in a 37°C incubator with daily observations of the cytopathic effect.	Obtained 23 isolates from different specimen types (12 from OP, nine from NP, and two from SP).	Specimens with high copy numbers of the viral genome, indicative of higher viral load, were more likely to be culturable.
Jeong 2020 [W10]	Naso/oropharyngeal swabs, saliva, urine, and stool	5 patients	Specimens positive by qPCR were subjected to virus isolation in Vero cells. Urine and stool specimens were inoculated intranasally in ferrets and they evaluated the virus titers in nasal washes on 2, 4, 6, and 8 days post-infection (dpi). Immunofluorescence antibody assays were also done.	Naso/ oropharyngeal saliva, urine and stool Specimens were collected between days 8 to 30 of the clinical course. Viable SARS-CoV-2 was isolated from 1 naso / oropharyngeal swab. Ferrets inoculated with patient urine or stool were	Viral loads in urine, saliva, and stool specimens were almost equal to or higher than those in naso / oropharyngeal swabs. After symptom resolution, patients shed viable virus in their saliva and urine up to day 15 of illness.

				infected. SARS-CoV-2 was isolated from the nasal washes of the 2 urine-treated ferrets and one stool-treated ferret	
Kim 2020 [W11]	Unclear. Possibly 323 serum 247 urine and 129 stool specimens	74 COVID-19 hospital patients	RT-PCR was performed on the target genes were E and RdRp. Cell culture was performed in a Level III facility by inoculum into CaCo-2 cell line after stabilisation at 4C and harvested after 5 days and the supernatant after centrifugation was re-inoculated for another 5 days and assessed with RT-PCR.	No viral growth was detected in any specimen despite a positive RT-PCR very soon after admission	
Korean CDC 2020 [W12]	Respiratory swab specimens for individuals testing positive after having previously tested positive, then negative.	108 specimens	Methods not reported	0 / 108 respiratory specimens	This report does not report the laboratory methods used.
Kujawski 2020 (for The COVID-19 Investigation Team) [W13]	Nasopharyngeal (NP), oropharyngeal (OP), stool, serum and urine specimens	12 patients had initial respiratory specimens collecte	SARS-CoV-2 real-time PCR with reverse transcription (rRT–PCR) cycle threshold (Ct) values of virus isolated from the first tissue culture passage were 12.3 to 35.7 and for one patient, virus isolated from tissue culture passage 3 had a titer of 7.75 × 106 median tissue culture infectious dose per ml; these data were likely more reflective of growth in tissue culture than patient viral load.	Viral culture was attempted on initial respiratory specimens from 9 patients and was successful in all 9, including 2 patients who not hospitalized	Viable SARS-CoV-2 was cultured at day 9 of illness (patient 10), but was not attempted on later specimens. SARS-CoV-2 rRT–PCR Ct values of virus isolated from the first tissue culture passage were 12.3 to 35.7.

					Mean Ct values in positive specimens were 17.0 to 39.0 for NP, 22.3 to 39.7 for OP and 24.1 to 39.4 for stool. All blood and urine isolates were negative. Ct values of upper respiratory tract specimens were lower in the first week of illness than the second in most patients.
L'Huillier 2020 [W14]	Nasopharyngeal swabs in 638 patients aged less than 16 years in Geneva Hospital	23 (3.6%) tested positive for SARS CoV-2 - median age of 12 years (range 7 days to 14.9 years) [1-4]	Observation of cytopathic effect on days 2,4, and 6 of inoculum in Vero cells in two passages.	12 (52% of PCR positive)	Ct was around 28 for the children whose specimens grew viable viruses
La Scola 2020 [W15]	Naso pharyngeal swabs or sputum specimens Only Naso pharyngeal specimens from the	183 (4384 specimens from 3466 patients) [not reported]	From 1,049 specimens, 611 SARS-CoV-2 isolates were cultured. 183 specimens testing positive by RT-PCR (9 sputum specimens and 174 nasopharyngeal swabs) from 155 patients, were inoculated in cell cultures. SARS-CoV-2. RNA rtPCR targeted the E gene. Nasopharyngeal swab fluid or sputum specimen were filtered and then inoculated in Vero E6 Cells. All specimens were inoculated	Of the 183 specimens inoculated in the studied period of time, 129 led to virus isolation. Of these 124 specimens had detectable cytopathic effect	There was a significant relationship between Ct value and culture positivity rate: specimens with Ct values of 13–17 all had positive culture. Culture positivity rate decreased progressively according to Ct values to 12% at 33 Ct.

	subsequent Jaafar et al letter.		between 4 and 10 h after sampling and kept at + 4 °C before processing. After centrifugation they were incubated at 37 °C. They were observed daily for evidence of cytopathogenic effect. Two subcultures were performed weekly and scanned by electron microscope and then confirmed by specific RT-PCR targeting E gene.	between 24 and 96 h The letter by Jaafar et al adds that 1941 SARS-Cov-2 30 isolate cultures were positive out 3 790 inoculated specimens. These could be seen after the first inoculation or up to 2 blind subcultures. At at Ct of > 34 2.6% of specimens yielded a positive culture.	No culture was obtained from specimens with Ct > 34. The 5 additional isolates obtained after blind subcultures had Ct between 27 and 34, thus consistent with low viable virus load.
Ladhani 2020 [W16]	Naso pharyngeal swabs	87 [Residents post, pre and symptomatic, 5 (6 to 3) 4 (2 to 11) 7 (10 to 4). Staff post, pre and symptomatic 7 (9 to 4) 3 (2-5) 5 (9 to _3)]	All SARS-CoV-2 positive specimens with a Ct value of <35 were incubated on Vero E6 mammalian cells and virus detection was confirmed by cytopathic effect (CPE) up to 14 days post-inoculation. Whole genome sequencing (WGS) was carried out on all RT-PCR positive specimens	87	Ct values < 35 Higher Ct values (lower virus load) specimens were associated with decreasing ability to recover infectious virus from 100% (2/2) with Ct <20.00 to 17.0% (9/53) with Ct 30.00_34.99 (x2 for trend, P<0.001)
Lu 2020 [W17]	87 cases testing “re-positive” at RT-PCR	619 hospital discharges of which tested positive after	137 swabs and 59 serum specimens from 70 “re-positive” cases to assess the immunological and virologic characteristics of the SARS-CoV-2 “re-positive” cases. From 23 January, hospital	No cultures were positive	“Re-positive” cases are unlikely to be infectious as no intact RNA single helix was detected or viral

	137 swabs (51 nasopharyngeal, 18 throat and 68 anal)	discharge	dischargees followed a strict isolation protocol living (for example) in single dedicated hotel rooms and went home only when nucleic acid tests were negative on both respiratory tract and digestive tract specimens. Specimens (nasopharyngeal, throat and anal swabs), were collected for RT-PCR diagnosis at 7 and 14 days after discharge. Culture was carried out by inoculating Vero E6 cells with patient specimen. CPE were observed daily at 7 days with a second round of passage. RT-PCR diagnosis was carried out on RNA using three RT-PCR kits to conduct nucleic acid testing, in an attempt to avoid false negatives. Ct varied from 29 to 39 depending on gene and kit		isolated grew. Prolonged detection of viral RNA is a challenge for public health interventions targeted at isolating infectious cases. “Re-positive” discharged cases are caused by intermittent shedding of cells containing remnant RNA.
Perera 2020 [W18]	68 specimens: nasopharyngeal aspirates combined with throat swab (n=49), nasopharyngeal aspirate (n=2), nasopharyngeal swab combined with throat swab (n=3), nasopharyngeal swab (n=2), sputum (n=11) and saliva	35 patients, 32 with mild disease [1 to 67 days]	Specimens were tested for sgRNA with ≥5 log10 N gene copies per ml. The complementary DNA obtained was subjected to PCR (40 cycles). Vero E6 cells were seeded and incubated for 24 hours in a CO2 incubator. The culture medium was removed and 125 µL of the clinical specimen in virus transport medium diluted and was inoculated into 2 wells. After 2 hours incubation in a CO2 incubator at 37°C, the plates were incubated at 37°C in a CO2 incubator. A specimen (100 µL) of supernatant was specimend for a quantitative real-time RT-PCR at 0 and 72 hours post inoculation. At 72 hours, cells were scraped into	Virus was isolated from 16 specimens for 16 patients out of a total of 35 specimens	Culturable SARS CoV-2 and sub-genomic RNA (good indicator of replication) was rarely detectable beyond 8 days after onset of illness although virus RNA by RT-PCR remained for up to 70 days.

	(n=1).		the supernatant and transferred onto fresh cells in 24-well plates and monitored for an additional 72 hours. A final quota of cells was collected for quantitative real-time RT-PCR. Cells were observed for cytopathic effect daily and harvested for testing if 25%–50% of cells showed a cytopathic effect.		
Qian 2020 [W19]	Rectal tissue obtained from a surgical procedure was available.	1 [1 to 3 days post op]	Ultrathin sections of tissue fixed in epoxy resin on formvar-coated copper grids were observed under electron microscope under 200kV. Immunohistochemical staining was used to establish expression and distribution of SARS-CoV-2 antigen.	1	No culture performed. Visualisation of virions in rectal tissue and detection of SARS-CoV-2 antigen in the rectal tissue.
Santarpia 2020 ([W20]	Windowsill and air, mean 7.3 specimens per room. The percentage of PCR positive specimens from each room was 40% -100%	13 patients [days 5 to 9 and day 18 of isolation in a quarantine unit]	Vero E6 cells were used to culture virus from environmental specimens. The cells were cultured in Dulbeccos's minimal essential medium (DMEM) supplemented with heat inactivated fetal bovine serum (10%), Penicillin/Streptomycin (10,000 IU/mL &10,000 µg/mL) and Amphotericin B (25 µg/mL).	Possibly 2 with weak cyotopathic effect	Isolates were from days 5 and 8 of occupancy of hospital/isolation rooms
Singanayagam 2020 [W21]	324 specimens: nose, throat, combined nose-and throat and nasopharyngeal swabs and aspirates	253 positive case [-10 to 60 days]	Vero E6 cells were inoculated with clinical specimens and incubated at 37 °C, 5% CO2. Cells were inspected for cytopathic effect daily up to 14 days. Presence of SARS-CoV-2 was confirmed by SARSCoV-2 nucleoprotein staining by enzyme immunoassay on infected	133 (41%) specimens (from 111 cases)	RT-PCR cycle threshold values correlate strongly with cultivable virus i.e. likelihood of infectiousness. Median Ct of all 324 specimens was 31.15. Probability of culturing virus

			cells.		declines to 8% in specimens with Ct > 35 and to 6% 10 days after onset and was similar in asymptomatic and symptomatic persons. Asymptomatic persons represent a source of transmissible virus but there is no difference in Ct values and culturability by age group.
Wang 2020 [W22]	Bronchoalveolar fluid, sputum, feces, blood, and urine specimens from hospital in-patients with COVID-19	1,070 specimens collected from 205 patients with COVID-19	rRT-PCR targeting the open reading frame 1ab gene of SARS-CoV-2; cycle threshold values of rRT-PCR were used as indicators of the copy number of SARS-CoV-2 RNA in specimens with lower cycle threshold values corresponding to higher viral copy numbers. A cycle threshold value less than 40 was interpreted as positive for SARS-CoV-2 RNA.	Live SARS-CoV-2 was observed in the stool specimen from 2 patients who did not have diarrhea.	The details of how the specimens were cultured were not reported.
Wölfel 2020 [W23]	Saliva, nasal swabs, urine, blood and stool	9 patients [2 to 4 days]	The average virus RNA load was 6.76 × 105 copies per the whole swab until day 5, and the maximum load was 7.11 × 108 copies per swab. The last swab specimen that tested positive was taken on day 28 after the onset of symptoms.	Yes in respiratory specimens, and indicative in stool	

Xiao F SJ 2020 [W24]	Esophageal, gastric, duodenal, and rectal tissues were obtained from 1 COVID-19 patients by endoscopy.	1 plus an unknown additional number of fecal specimens from RNA-positive patients.	Histological staining (H&E) as well as viral receptor ACE2 and viral nucleocapsid staining were performed.	1/1 RNA-positive patient. Positive staining of viral nucleocapsid protein was visualized in the cytoplasm of gastric, duodenal, and rectum glandular epithelial cell, but not in esophageal epithelium of the 1 patient providing these tissues. Additionally, positive staining of ACE2 and SARS-CoV-2 was also observed in gastrointestinal epithelium from other patients who tested positive for SARS-CoV-2 RNA in feces, results not shown.	Total specimen numbers are not reported.
Xiao F 2020 [W25]	Serial feces specimens collected from 28 hospitalised COVID-19 patients: 3 specimens from 3 RNA-positive patients were tested for possible viral culture.	3, one patient admitted day 7 post onset	Inoculation of Vero 6 cells. Cycle threshold values for the fecal specimen were 23.34 for the open reading frame 1lab gene and 20.82 for the nucleoprotein gene. A cytopathic effect was visible in Vero E cells 2 days after a second-round passage. The researchers negatively stained culture supernatant and visualized by transmission electron microscopy. Viral particles that were visible were spherical and had distinct surface spike protein projections, consistent with a previously published SARS-CoV2 image.	Infectious virus was present in faeces from two cases)	Selection of specimens is not entirely clear.

Yao 2020 [W26]	Sputum (n=7), stool (n=3) and one nasopharyngeal specimen	11 patients admitted to hospital: 9 classified as serious or critical, 1 moderate, 1 mild symptoms [0 to 16 days]	The specimens of the 11 patients involved in this study were collected during the early phase of the Covid-19 break out in China, dates ranging from 2nd of January to the 2nd of April 2020. All except one of the patients had moderate or worse symptoms. Three patients had co-morbidities and one patient needed ICU treatment. Seven patients had sputum specimens, one nasopharyngeal and three had stool specimens The specimens were pre-processed by mixing with appropriate volume of MEM medium with 2% FBS, Amphotericin B, Penicillin G, Streptomycin and TPCK-trypsin. The supernatant was collected after centrifugation at 3000 rpm at room 434 temperature. Before infecting Vero-E6 cells, all collected supernatant was filtered using a 435 0.45 µm filter to remove cell debris etc. Vero-E6 cells were infected with 11 viral isolates and quantitatively assessed their viral load at 1, 2, 4, 8, 24, and 48 hours post-infection (PI) and their viral cytopathic effects (CPE) at 48 and 72 hours PI and examined whether the viral isolates could successfully bind to Vero-E6 243 cells as expected. Super-deep sequencing of the 11 viral isolates on the Novaseq 6000 platform was performed.	11 specimens taken up to 16 days from admission to hospital.	Cultured viruses were inoculated in Vero cells. At 8 hours post-infection there was a significant decrease in Ct value (increases in viral load) for five isolates. At 24 hours significant decreases in the Ct values for all of the viral isolates were observed. Mutations of the viruses are also reported

Young 2020 [W27]	Naso pharyngeal swabs, stool, fresh urine	152 of 74 patients	Material from nasopharyngeal swabs was inoculated in Vero-E6 cells in a Level 3 laboratory. Urine and stool specimens were collected and transported fresh for virus culture but stools were filtered before inoculation. Cells were cultured at 37C for seven days or less if cytopathic effect (CPE) was observed by day 4 and confirmed by PCR.	21 naso pharyngeal specimens from 19 (14%) patients	No virus was isolated when the PCR cycle threshold (Ct) value was >30 or >14 days from symptom onset. Urine and stool specimens at admission did not grow virus
Zhang 2020 [W28]	Stool	Unknown [not reported]	Vero cells were used for viral isolation from stool specimens of Covid-19 patients. A 2019-nCoV strain was isolated from a stool specimen of a laboratory-confirmed COVID-19 severe pneumonia case, who experienced onset on January 16, 2020 and was specimend on February 1, 2020. The interval between sampling and onset was 15 days. The full-length genome sequence indicated that the virus had high-nucleotide similarity (99.98%) to that of the first isolated novel coronavirus isolated from Wuhan, China. In the Vero cells, viral particles with typical morphology of a coronavirus could be observed under the electron microscope.	1	We do not know what influenced successful virus culture e.g. methods optimal, or concentration of virus optimal. More information needed.
Zhou 2020 [W29]	218 surface specimens 31 air specimens	7 areas of large London hospital	RT-PCR with primers and probes for the envelope (E) gene. Duplicate PCR was carried out and specimens were considered positive if both duplicates had Ct< 40.4, or suspect if one of the two have Ct<40.4 (equivalent to one genome copy. For culture Vero E6 and Caco2 cells were used from air and environmental	No cultures were positive	The pre-defined cycle threshold cut off was too high

			specimens using a method adapted from one previously used to culture influenza virus. On day 0 and after 5-7 days, cell supernatants were collected, and RT-qPCR to detect SARS-CoV-2 performed as described above. Specimens with at least one log increase in copy numbers for the E gene (reduced Ct values relative to the original specimens) after 5-7 days propagation in cells compared with the starting value were considered positive by viral culture.		

Key: STT = symptom onset to test date.

Table 2. Quality of included studies

Study	Description of methods and sufficient detail to replicate	Sample sources clear	Analysis & reporting appropriate	Is bias dealt with	Applicability
Ahn 2020 [W1]	Yes	Yes	Yes	Partly	Unclear
Andersson 2020 [W2]	Yes	Yes	Yes	Partly	Yes
Arons 2020 [W3]	Yes	Yes	yes	Yes	Unclear
Basile 2020 [W4]	Yes	Yes	Yes	Unclear	unclear
Borczuk 2020 [W5]	Yes	Yes	Yes	Yes	Unclear
Brown 2020 [W6]	Yes	Yes	Yes	Unclear	Unclear
Bullard 2020 [W7]	Yes	Yes	yes	unclear	Unclear
Gniazdowski [W8]	Yes	Yes	Yes	Unclear	Unclear
Huang 2020 [W9]	yes	Yes	Yes	Unclear	Unclear
Jeong 2020 [W10]	Yes	Yes	Yes	No	Unclear
Kim 2020 [W11]	No	No	No	Unclear	Unclear
Korean CDC [W12]	No	Partly	Partly	No	Unclear
Kujawski 2020 [W13]	Yes	Yes	Yes	Unclear	Unclear

L'Huillier 2020 [W14]	Yes	Yes	Yes	Unclear	Unclear
La Scola 2020 [W15]	Yes	Yes	Yes	Unclear	Unclear
Ladhani 2020 [W16]	Yes	Yes	Yes	Yes	Likely
Lu 2020 [W17]	Yes	Yes	Yes	Partly	Yes
Perera 2020 [W18]	Yes	Yes	Yes	Unclear	Unclear
Qian Q 2020 [W19]	Yes	Yes	Yes	Unclear	Unclear
Santarpia 2020 [W20]	Yes	Yes	Yes	Unclear	Unclear
Singanayagam [W21]	Yes	No	Yes	Unclear	Unclear
Wang W 2020 [W22]	No	Yes	Yes	No	Unclear
Wölfel 2020 [W23]	Yes	Yes	Yes	Unclear	Unclear
Xiao FSJ 2020 [W24]	No	Yes	Yes	No	Unclear
Xiao F 2020 [W25]	Yes	Yes	Yes	No	Unclear
Yoa H 2020[W26]	Yes	Yes	Yes	Unclear	Unclear
Young 2020 [W27]	Yes	Yes	Yes	Yes	Yes
Zhang 2020 [W28]	Partly	Yes	yes	No	Unclear
Zhou 2020 [W29]	Yes	Yes	Yes	Unclear	Unclear

Table 3. Duration of **detectable SARS-CoV-2 RNA** in the included studies.

Study	**Duration of detectable SARS-CoV-2 RNA as assessed by PCR**	**Comments on the clinical course**
Bullard [w7]	Specimens included in this study included those positive for SARS-CoV-2 by RT-PCR from day of symptom onset (Day 0) up to 21 days post symptom onset.	SARS-CoV-2 Vero cell infectivity of respiratory specimens from SARS-CoV-2 positive individuals was only observed for RT-PCR Ct < 24 and symptom onset to test of < 8 days.
Gniazdowski [w8]	Patients that received repeated testing with longitudinal positive results were tested within a time frame that ranged from less than one day to more than 45 days	Four patients had infectious virus recovered from specimens collected in up to 22 days after the first positive result. Many patients who tested negative for SARS-COV-2 showed a subsequent positive result
Jeong [w10]	Five positive-PCR patients, day 8 to day 30 after symptom onset.	Viable SARS-CoV-2 was demonstrated in saliva, urine and stool specimens from COVID-19 patients up to days 11-15 of the clinical course.

Korean CDC [w12]	On average, it took 45 days (range: 8 to 82 days) from the initial symptom onset date to testing re positive after discharge. (Based on 226 cases symptomatic at the time of initial confirmation)	This may indicate duration of viral RNA detection over a long period of time and inconsistently. These data may not be comparable with information from studies specifically observing the duration of viral RNA detection as an outcome. Time to retesting positive via PCR is reported, among this specific group of individuals who retested positive by PCR.
Kujawski (for The COVID-19 Investigation Team) [w13]	Duration of SARS-CoV-2 detection by RT-PCR was 7 to 22 days	First 12 identified patients in the US. Respiratory specimens collected between illness days 1 to 9 (median, day 4) All patients had SARS-CoV-2 RNA detected in respiratory specimens, typically for 2 to 3 weeks after illness onset. Mean duration of fever was 9 days. Two patients received a short course of corticosteroids.
Singanayagam [w21]	SARS-CoV-2 viral load identified that the level of SARS-CoV-2 RNA in the URT was greatest around symptom onset, steadily decreased during the first 10 days after illness onset and then plateaued up to day 21	Probability of culturing virus declined to 8% in specimens with Ct > 35 and to 6% 10 days after onset;
Xiao F SJ [w24]	The viral load was higher in feces than in respiratory specimens collected at multiple time points (17–28 days after symptom onset)	Isolation of virus from feces specimens collected at later time points was not successful, although results for virus RNA remained positive, indicating only RNA fragments, not infectious virus, in feces of this patient collected at later time points of disease onset.
Xiao F w25	The duration time of positive stool results ranged from 1 to 12 days.	17 (23%) patients continued to have positive results in stool after showing negative results in respiratory specimens.

Young w27	SARS-CoV-2 RNA was detectable from nasopharyngeal swabs by PCR up to 48 days after symptom onset.	Mean duration of viral RNA detection by PCR was 16.7 days (95% CI 15.2-18.3). C Cessation of viral RNA detection by PCR occurred in 4% by day 7, 30% by day 14, 78% by day 21 and 91% by day 28. There were no differences by disease severity No virus was isolated when the PCR cycle threshold (Ct) value was >30 or >14 days after symptom onset.

Table 4: Relationship of PCR Cycle threshold and Log ^{10} copies to Positive Viral Culture

	Specimen				Cycle Threshold			Log ^{10} copies			
Study	RT-PCR SARS-CoV-2 positive specimens (n)	Viral Culture growth (n)	No growth (n)	Gene fragment sampled on PCR Test	Positive culture Ct value	Negative culture Ct Value	No growth in specimens based on Ct	Log ^{10} copies positive culture (unless otherwise stated)	Log ^{10} copies negative culture	No growth based on log copies	ORs for Viral Culture
Basile 2020 [w4]	234	56	178	E, RdRp, N, M, and ORF1ab for ICU patients;	25.01	27.75	Ct >32 with the N gene target [3]				
Brown 2020 [w5]	23	1	22	RdRp, E, and N	26.16	35.16 ± SEM 0.63	Ct >26.2				
Bullard 2020 [w7]	90	26	64	E gene	17 [16-18]	27 [22-33]	Ct > 24				OR 0.64 (95%CI 0.49 to 0.84, p<0.001) for every one unit increase in Ct.
Gniazdowski 2020 [w8]	132	47	85	S, Nsp 2	mean 12.8 ± 3.4 median 18.17	mean 27.1 ± 5.7 median 27.5	Ct ε 23 yielded 8.5% of virus isolates				
Huang 2020	60	23	34	Nsp 12	mean 23.9 ±	mean 29.26 ±	Ct >31.47	mean 7.37 ±	mean 5.98 ±		

[w9]					SEM 0.78	SEM 0.78		SEM 0.20	SEM 0.18		
		23	37	E	mean 22.39 ± SEM 0.75	mean 28.92 ± SEM 0.65	Ct >31.46	mean 8.21 ± SEM 0.18	mean 6.62 ± SEM 0.16		
		21	31	N	mean 27.29 ± SEM 0.77	mean 31.49 ± SEM 0.59	Ct >35.2	mean 7.87 ± SEM 0.21	mean 6.70 ± SEM 0.17		
L'Huillier 2020 [w14]	23[4]	12	11					mean 7.9×10^8 IQR $4.7\cdot10^6$ - $1.0\cdot10^9$	mean 5.4×10^7 IQR 4.2×10^3– 1.8×10^6		
La Scola 2020 (Jaafar 2020) [w15]	611 (3790)	129(1941)	482 (1849)	E			Ct ε 34 (2,6% positives)				
Ladhani 2020 [w16]	87	31	56	ORF1ab	100% cultures (2/2) with Ct <20.00 to 17.0% (9/53) with Ct 30.00-34.99	Cutoff >35					
Perera 2020 [w18]	68	16	52	N				7.5 [2]	3.8	<5.0	
Singanayagam 2020 [w21]	324	133	191	Unclear			Ct > 35 probability of no growth was 8.3% (95% CI: 2.8%–18.4%) [1]				OR 0.67 for each unit increase in Ct value (95% CI: 0.58–0.77)

Exhibit 5

Wölfel 2020 [w23]	45	9	36	E, Subgenomic mRNA.						
Young 2020 [w27]	100	21	79	N, S, and ORF1ab	28.2 (24.3 to 33.3	>30				

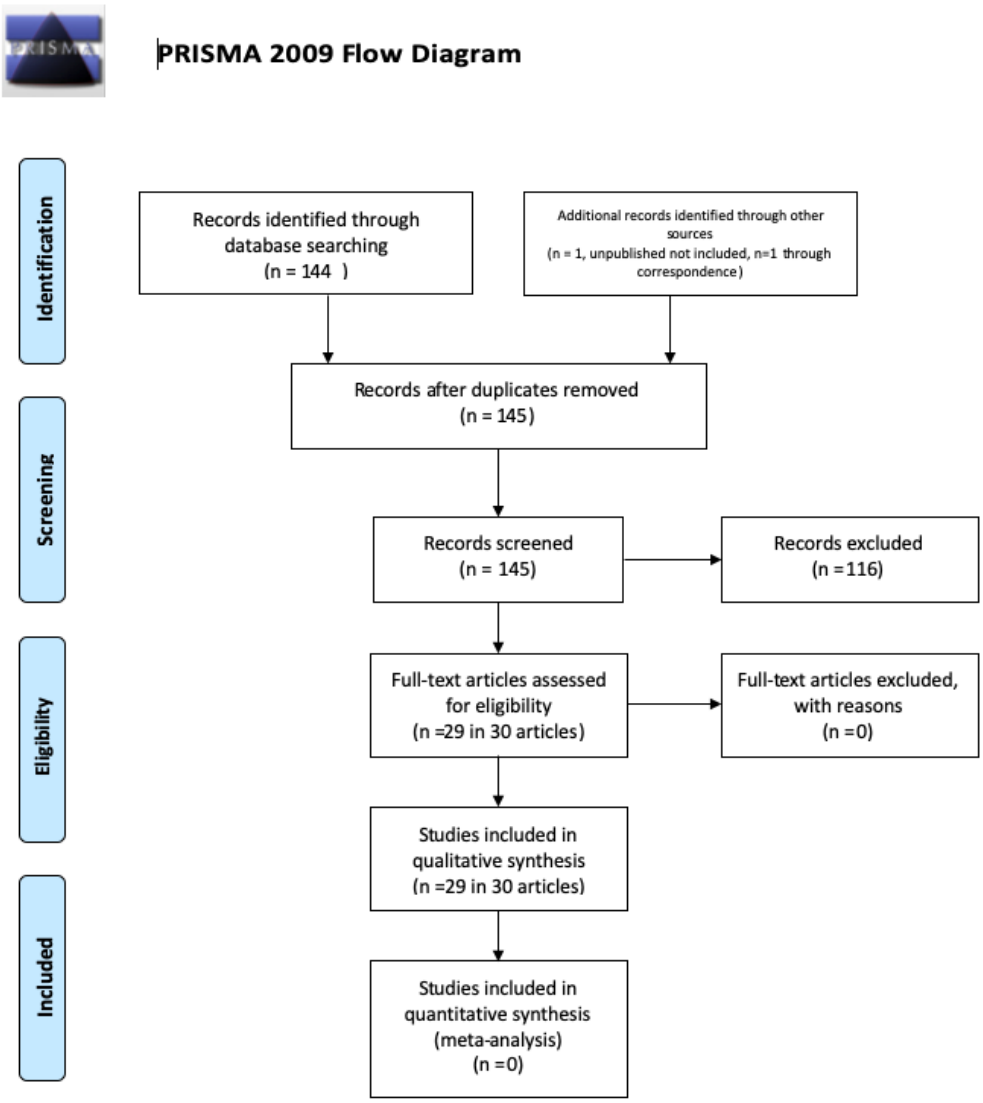
1 All above CT (n=5) 35 were symptomatic

2. Of the 16 culture-positive specimens, 15 (94%) had viral RNA load >6 log10 copies/mL ($p<0.01$). All of them were collected within the first 8 days of illness

3. no CPE visualised but a decrease in Ct values between the Ct of the original clinical specimen PCR ($Ct_{specimen}$) and the terminal culture (day four) supernatant PCR ($Ct_{culture}$) of ≥3 (equivalent to a 1 log increase in virus quantity) i.e. $Ct_{specimen} - Ct_{culture} \geq 3$ = culture positive. The authors hypothesized that a $Ct_{specimen}$ minus $Ct_{culture}$ <3 was due to residual inoculated clinical specimen and not replicating virus.

4.23 SARS-CoV-2–infected children

Figure 1



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

Exhibit 5